Proteinase K

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I.U.B.: 3.4.21.14 ***Tritirachium* alkaline proteinase**

Proteinase K (PROK) is a serine endopeptidase with a broad spectrum of action, isolated from the filtrate of the fungus *Tritirachium album limber.*

Characteristics of Proteinase K from *Tritirachium album limber:*

Molecular weight: 28,900 daltons.

pH Optimum: 7.5 - 12, using denatured hemoglobin as substrate.

Stability: Although calcium ions do not affect the enzyme activity, they do contribute to its stability when present at a concentration of 1 - 5 ?moles. An interesting characteristic of proteinase K is that it retains its activity in the presence of sodium dodecyl sulphate (SDS) or urea. (0.5 - 1% SDS and 1 - 4 M urea). Raising the temperature of the reaction from 37°C to 50°C - 60°C can increase the activity several folds. A special feature of proteinase K is its ability to digest native proteins, thereby inactivating enzymes such as DNase and RNase without recourse to a denaturation process.

Specificity: In addition to cleavage of peptide bonds, it is able to catalyze peptide amide hydrolysis. Proteinase K is inactivated by diisopropyl fluorophosphate (DFP) or phenyl methane sulphonyl fluoride (PMSF). Chelating agents such as citrate and EDTA have no affect on the enzyme activity.

Application: Proteinase K is very useful in the isolation of highly native, undamaged DNAs or RNAs, since most microbial or mammalian DNases and RNases are rapidly inactivated by the enzyme, particularly in the presence of 0.5 - 1% SDS.

Storage: Store at 2 - 8°C.

Worthington proteinase K is supplied as a highly purified lyophilized powder. It is tested to be free of DNase and RNase.

Assay

Method: Proteinase K hydrolyzes hemoglobin denatured with urea, and liberates Folin Postive amino acids and peptides, which are determined as tyrosine equivalents. 1 unit releases 1 ? mole of Folin positive amino acid in 10 minutes at 37°C, pH 7.5, using denatured hemoglobin as substrate.

Reagents

0.05 N HCl - Dilute 0.82 ml conc. HCl to 200 ml with reagent grade water.
0.5 M NaOH - Dissolve 4.0 gm NaOH in 200 ml reagent grade water.
Buffer-Substrate - Dissolve 2.0 gm hemoglobin in 35 ml reagent grade water, add 36.0 gm urea and 16

ml 0.5 M NaOH. Stir for 30 - 60 minutes at room temperatue. Add 0.618 gm boric acid and stir. Adjust the pH to 7.5 with 5 N HCl and q.s. to 100 ml.
Tyrosine standard (2.5 nmol/L) - Dissolve 45.3 mg tyrosine in 100 ml of 0.05 N HCl.
0.3 M Trichloroacetic acid - Dissolve 9.8 gm trichloroacetic acid in 200 ml reagent grade water.
Folin Reagent - Add 10 ml Folin-Ciocalteus Phenol Reagent to 20 ml reagent grade water.

Enzyme

Dissolve 10 mg lyophilized material in 1 ml reagent grade water. Prepare a 1:1000 dilution with water immediately before use.

Procedure

Label clear glass test tubes for blank, standard, and test. Add 2.5 ml buffer-substrate and incubate for 5 minutes at 37°C. Start reaction by adding 0.2 ml tyrosine standard to the standard tube, 0.2 ml of sample to the test, and 0.2 ml of 0.05 N HCl to the blank. Incubate for 10 minutes at 37°C. Stop reaction by the addition of 5.0 ml trichloroacetic acid. Mix, then add 0.2 ml of sample to the blank and standard, and add 0.2 ml of 0.05 N HCl to the test. Mix and let stand for 10 minutes at room temperature, filter and pipette into test tubes 1.0 ml of filtrate, 2.0 ml of 0.5 N NaOH, and 0.6 ml of Folin Reagent. Mix well. Let stand for 15 minutes and read A_{578} nm.

Calculation

$$\text{Units/mg} = \frac{0.5\mu\text{moles tyrosine}}{0.2\text{ml} \times 10\text{ min}} \times \frac{(A_{578}\text{ of sample} - A_{578}\text{ of blank})}{A_{578}\text{ of standard}} \times \text{Dilution}$$

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